somewhat less than that of IbpB and presence of both IbpA and IbpB has synergistic effect on holdase activity; as measured from the inhibition of aggregation of DTT-denatured insulin B chains in the presence of IbpA, IbpB and IbpA+IbpB (IbpAB) and b) both IbpA and IbpB has in vitro protein folding property, as measured from the gain in activity of the denatured alkaline phosphatase (denatured either by guanidine hydrochloride or heat) after incubation with IbpA, IbpB, or IbpAB for a prolonged period of about 6 hr. To conclude briefly, it can be stated that E. coli small heat-shock proteins IbpA and IbpB have both holding and folding chaperone activities on denatured proteins, the activities being maximum at 500C and the effects are synergistic when IbpA and IbpB act in combination.

1144-Pos Board B54

The Disk Membrane Bilayer Enhances Bovine Rhodopsin Kinetic Stability

Arlene D. Albert, Scott C. Corley.

The photoreceptor, rhodopsin is a kinetically stable membrane protein. This G-protein coupled receptor consitiutes greater than 90 percent of the membrane proteins of rod outer segment disk membranes. Therefore biophysical studies of rhodopsin can be carried out in its native membrane. The role of the native bilayer in maintaining the kinetic stability rhodopsin was investigated. The disks were systematically disrupted by the detergent, octyl-β-D-glucopyranoside (OG). Rhodopsin kinetic stability was examined under sub-solubilizing conditions in which the bilayer was partially disrupted as well as fully solubilizing conditions in which rhodopsin was largely delipidated. In differential scanning calorimetry (DSC) studies rhodopsin exhibited an irreversible scan rate dependent endothermic transition and a scan rate dependent exothermic transition at all stages of solubilization. The endothermic Tm decreased and the exothermic Tm increased as the OG partitioned into the bilayer. There was little change once the fully solubilized stage was achieved even though phospholipids were present in the mixed micelles. The activation energy of denaturation (Eact) was calculated at each stage of membrane disruption from the scan rate dependence of the Tm. The endothermic Eact decreased rapidly in the sub-solubilizing phase, but not once the membrane was fully solubilized. The Eact determined from the rate of thermal bleaching was in agreement with the DSC data. The degree of solubilization had no effect on the exothermic transition Eact. The calorimetric enthalpy (ΔHcal) was independent of the extent of solubilization. The thermal transition broadened during the sub-solubilizing phase suggesting increased protein motional freedom. There was no further broadening in the fully solubilized stage. These results indicate that the phospholipids present in detergent micelles do not kinetically stabilize the protein. The membrane bilayer enhances the kinetic stability rhodopsin by increasing the energy barrier to denaturation.

1145-Pos Board B55

Size and Shape of Crowders Affect the Folding Landscape of Cytochrome C

Qian Wang, Antonios Samiotakis, Margaret Cheung.

We use computer simulation to investigate the effects of synthetic crowders on cytochrome c, a small single-domain protein with a cofactor heme. The folding energy landscape of cytochrome was computed in the presence of crowders with various sizes and shapes by using a coarse-grained protein model and the structure-based (Go-like) interactions. Our results demonstrated that given the same volume fraction of crowders, the stability of a folded protein inversely increases with the radius of crowders. In addition, a crowder with an anisotropic geometry imposes a greater stabilizing effect on the folded protein than isotropic crowders. This is in agreement with the predictions by the scaled particle theory. In addition, the distribution of contact formation between heme and cytochrome c protein was found to be varied by different types of crowders, demonstrating that the geometry of crowders may be one of the key factors for tuning heme-protein contact formation under cell-like conditions. Prospects of mixed crowders will be presented.

1146-Pos Board B56

Kinetic Models of Enhanced Sampling Methods

Edina Rosta, Gerhard Hummer.

We present kinetic models of enhanced sampling methods such as replica exchange and simulated tempering. With these models we derive analytical expressions for the statistical error and computational efficiency of the sampling methods. As a specific example, we consider two-state protein folding. A main result is that with comparable computational resources used, the relative efficiency of replica exchange molecular dynamics (REMD) and molecular dynamics (MD) simulations is given by the ratio of the number of transitions between the two folding states averaged over all replicas at the different temperatures, and the number of transitions at the single temperature of the MD run. This formula applies if replica exchange is frequent, as compared to the transition times. For simulated tempering (ST) simulations, we obtain a relation for the efficiency that is derived for the limit in which changes in the ST temperature are fast compared to the two-state transitions. In this limit, ST is most efficient. Our expression for the maximum efficiency gain of ST simulations is essentially identical to the corresponding expression derived for replica exchange. Implications on actual protein folding simulations will be discussed.

1147-Pos Board B57

Joint Experimental and Theoretical Investigation of the Interaction Between Antimicrobial Peptides, Gold Nanoparticles and Membranes

Julia Setzler, Yvonne Klapper, Annika Leifert, Timo Strunk, Anne Ulrich, Ulrich Simon, Roland Benz, **Wolfgang Wenzel**.

Antimicrobial peptides and gold nanoparticles (AuNPs) are interesting novel classes of pharmaceutically active compounds. To understand and optimize their efficacy, interactions of these systems with biological membranes need to be characterized. Given the wide range of synthetic possibilities, either by sequence design (peptides) or size, composition and ligand shell (nanoparticles), computational methods may help designing active compounds with predictable membrane permeability. Here we investigate a range of implicit membrane models, as extensions of our implicit solvent force field PFF02, to understand details of experimentally observed membrane association properties of naturally occurring antimicrobial peptides, in particular Gramicidin A/S and ligand stabilized gold nanoparticles of different size. Membrane association and penetration were studied in black lipid membrane (BLM) experiments using DOPC or DiphPC/DiphPG and DiphPE, model membranes. For Gram A we observed the transient formation of individual pores in the experiments, which are rationalized by simulations showing the dimerization of the helical peptides in the membrane. For Gram S, in agreement with the modeling results, we observe only small, fluctuating currents. We also observe size selective membrane association of the gold nanoparticles, where membrane integration of nanoparticles of 15 nm diameter generated ion-selective currents, while smaller 1.4 nm particles did not show such effects.

1148-Pos Board B58

Folding Kinetics of Small Proteins Revealed by Tryptophan-Cysteine Contact Formation Experiments

Marco Buscaglia, Andrea Soranno, Troy Cellmer, Renato Longhi, Tommaso Bellini.

Spectroscopic probes sensitive to the formation intra-chain contacts are increasingly used to study the structural and dynamical properties of polypeptides. Quenching of the triplet state of tryptophan by close contact with cysteine enables the measure of contact formation rates without the need of extrinsic probes, thus being suitable for the study of natural proteins and peptides. We illustrate the use of this method to investigate the conformational dynamics of small two-state proteins and of beta-hairpin peptides in conditions close to native. The coexistence of folded and unfolded states can be revealed from the non-exponential relaxation of the excited triplet, enabling the characterization of both unfolded chain dynamics and folding kinetics. The comparison with protein fragments and model disordered peptides allows estimating the contribution of different chain regions to the folding process. Using this approach, we outline the kinetic pathway leading to the formation of the beta-hairpin structure of the C-terminal fragment of protein GB1, revealing the presence of misfolded states, as proposed in recent computational studies.

1149-Pos Board B59

Interplay Between Secondary and Tertiary Structure Formation in Protein Folding Cooperativity

Tristan Bereau, Michael Bachmann, Markus Deserno.

Protein folding cooperativity is defined by the nature of the finite-size thermodynamic transition exhibited upon folding: two-state transitions show a free-energy barrier between the folded and unfolded ensembles, while downhill folding is barrierless. A microcanonical analysis, where the energy is the natural variable, has proved to be better suited than its canonical counterpart to unambiguously characterize the nature of the transition. Replica-exchange molecular dynamics simulations of a high-resolution coarse-grained model allow for the accurate evaluation of the density of states in order to extract precise thermodynamic information and measure its impact on structural features. The method has been applied to three helical peptides: a short helix shows sharp features of a two-state folder, while a longer helix and a three-helix bundle exhibit downhill and two-state transitions, respectively. Extending the results of lattice simulations and theoretical models, we have found that it is the interplay between secondary structure and the loss of non-native tertiary contacts that determines the nature of the transition. (Published in [Bereau, Bachmann, Deserno, J. Am. Chem. Soc. 132, pp 13129-13131 (2010)].)